AUTOMATED HUMAN AGE AT DEATH ESTIMATION SYSTEM FROM LONG BONES HISTOLOGY

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A thesis submitted in
fulfillment of the requirement for the award of the
Degree of Doctor of Philosophy in Electrical Engineering

Faculty of Electrical and Electronic Engineering
Universiti Tun Hussein Onn Malaysia

APRIL 2019

This humble work is dedicated to my beloved mother who taught me to accomplish through patience, determination and faith, my father, my sisters and brothers. Their love, prayers, support and motivation always inspire me to achieve my goals.

ACKNOWLEDGEMENT

"Exalted are You; we have no knowledge except what You have taught us. Indeed, it is You who is the Knowing, the Wise." (Al-Baqarah: 32)

First and foremost, I am grateful and indebted to Almighty Allah, the lord of creation, for letting me experience the beauty of life and providing me opportunity to conduct this study.

It is a genuine pleasure to express my deepest gratitude to my PhD supervisor Assoc. Prof. Dr. Muhammad Mahadi Abdul Jamil for his invaluable guidance and academic and psychological support. He continually and convincingly conveyed a spirit of adventure in regard to research and an excitement in regard to teaching. In the beginning, he helped in setting clear goals and then continuously provided a constructive feedback and skillful monitoring to organize the workflow of the study. The publications of this study and achievement of awards is the outcome of his intellectual directions and insight to the research. He also facilitated in getting scholarship for this work. Without his advice, motivation, expertise and understanding nature this research and dissertation would not have happened.

I am also grateful to Assoc. Prof. Dr. Faridah Mohd Nor for providing the bone samples through legal procedure. I would like to express my gratitude to Assistant Prof. Dr. Tahira Anwar Lashari for her valuable suggestions and support. I am also grateful to my best friend Hadi Abdullah, who have been helpful in every step and phase of my life. I am thankful to Dr Mohd Shamian Bin Zainal for his endless support during this study. I am grateful to my brother Dr. Mohd Ayaz khan for his guidance and support throughout my life, to my parents and elder siblings for giving me vision and motivation. And last but not the least, I am thankful to Office for Research, Innovation, Commercialization and Consultancy Management (ORICC) and University Tun Hussien Onn Malaysia for providing the opportunity to conduct this research.

ABSTRACT

Human age estimation at death from bone histology is a frequent and important requirement in forensic anthropology. Usually human age at death is estimated manually from bone histology or morphology. Manual methods of age estimation from bone histology involve three main phases that includes, analysis of variations in microscopic characteristics of bone with age, developing age regression equation based on the variation analysis and estimation of age using regression equation. However manual age at death estimation is not only tedious and time consuming process but also prone to observation variability and produce subjective results. Furthermore, there exists no digital database that can store the information of bone samples of Malaysian population. Hence it is vital to develop a histological automated system for age at death estimation to eliminate the problems of manual methods. This study presents the development of automated system for human age at death estimation from bone histology. Six histological and two morphological parameters were analyzed in 44 samples of long bones (humerus, radius, ulna, tibia, fibula and femur). First, the measurements and analyses were carried out using manual methods and then an automated system was developed to eliminate the problems of the manual process. The system assists in automatic measurements and calculations of bone histological parameters, analysis of parameters with age, developing regression equation and estimation of age. The automatic system also provides a digital database capable of storing the information of all parameters. The results of the system shows that histological parameters specifically percentage area covered by Haversian canals and mean Haversian canal area possess the highest correlation with age. Morphological parameters do not show significant correlation with age in Malaysian population. Age regression equation is developed with SEE of 8.3 years. The automatic system estimates age within 10 years of the actual ages for 89% of the samples. The automatic system is evaluated by seven forensic anthropologists and is considered effortless and acceptable for automatic age at death estimation from bone histology.

ABSTRAK

Anggaran usia kematian manusia menerusi histologi tulang adalah perkara yang penting dalam antropologi forensik. Pengiraan kaedah masa kematian manusia masih menggunakan cara manual morfologi. Kaedah tersebut melibatkan tiga fasa utama yang merangkumi, analisis variasi ciri-ciri mikroskopik usia tulang, persamaan regresi umur berdasarkan analisis variasi dan anggaran umur menggunakan persamaan regresi. Walau bagaimanapun, kaedah anggaran kematian menggunakan histologi tulang adalah menjemukan dan memakan masa akan tetapi terdedah kepada kepelbagaian pemerhatian yang subjektif. Selain itu, tiada pangkalan data sistematik digital yang menyimpan maklumat sampel tulang penduduk Malaysia. Oleh itu, adalah penting untuk membangunkan suatu sistem automatik histologi untuk menganggarkan umur kematian manusia dan menghapuskan masalah kaedah manual. Kajian ini membentangkan pembangunan sistem automatik penganggaran umur kematian manusia dari histologi tulang. Enam histologi dan dua parameter morfologi telah dianalisis menggunakan 44 sampel tulang panjang (humerus, radius, ulna, tibia, fibula dan femur). Pertama, pengukuran dan analisis telah dijalankan menggunakan kaedah manual beserta pembangunan sistem automatik. Sistem ini membantu pengiraan automatik parameter histologi tulang, analisis parameter untuk usia, persamaan regresi dan anggaran usia. Sistem automatik juga menyediakan pangkalan data digital yang mampu menyimpan maklumat semua parameter. Hasil dari sistem ini menunjukkan bahawa parameter histologi khususnya kawasan peratusan yang diliputi oleh terusan Haversian dan min kawasan Haversian memiliki korelasi tertinggi dengan parameter usia morfologi tidak menunjukkan korelasi yang ketara dengan usia penduduk Malaysia. Formula regresi umur yang dikembangkan oleh SEE adalah sebanyak 8.3 tahun. Sistem automatik menganggarkan usia dalam anggaran 10 tahun dari usia sebenar untuk 89% sampel. Sistem automatik yang telah dinilai oleh tujuh ahli antropologi forensik ialah dianggap mudah dan boleh diterima untuk anggaran usia kematian histologi tulang.

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LIST OF ABBREVIATIONS

2D	-	Two dimensions
3D	-	Three dimensions
µm	-	Micro meter
mm	-	Millimeter
A	-	Anterior
AL	-	Anterolateral
AM	-	Anteromedial
BMU	-	Basic multicellular unit
C	-	Celsius
CSI	-	Crime scene investigations
CT	-	Cortical thickness
DVI	-	Disaster victim identification
F	-	Fragments
GUI	-	Graphical user interface
H+	-	Hydrogen Ion
HCA	-	Haversian canal area
HCD	-	Haversian canal diameter
HCM	-	Mean Haversian canal area
HCN	-	Total Haversian canal number
Hcpar	-	Percentage Haversian canal area
HCR	-	Mean Haversian canal radius
HLC	-	Haversian lamella count
HP	-	Hyperparathyroidism
HUKM	-	Hospital University Kebangsaan Malaysia
L	-	Lateral
M	-	Medial
MCD	-	Medullar cavity diameter

Mpfb - Mean percent fragmental bone
Mpob - Mean percent osteonal bone
Mpub - Mean percent unremodeled bone
MSE - Mean squared error
N/A - Not attempted
NH - Non-haversian
NIH - National Institutes of Health
OC - Osteon count
OD - Osteon density
OI - Osteogenesis imperfecta
OP - Osteon population
OPD - Osteon population density
p - Significance value
P - Posterior
PC - Primary canal
PL - Posterolateral
PM - Posteromedial
RAP - Regional acceleratory phenomenon
RMSE - Root mean squared error
ROI - Region of interest
RS - Resorption spaces
SEE - Standard error of estimation
SPPS - Statistical Package for Social Sciences
UKM - University Kebangsaan Malaysia
UKMMC - University Kebangsaan Malaysia Medical Centre
VIAS - Volume Integration and Alignment System

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CHAPTER 1

INTRODUCTION

1.1 Background

The skeleton changes across the human life span. These variations are characterized by bone formation and growth throughout childhood, followed by a gradual loss of bone density. Loss of bone density in human skeleton begins in early adulthood and can accelerate significantly in older adults. Human skeleton have the capability to store the information of these variations that can be studied and analyzed even long after their death. Human skeletal remains offer significant source of information about the identity of a body, ancestry and the cause and time of death (Hadi, Muhammad & Amber, 2018). The identification of an individual from skeletal remains makes it of particular importance in case of decomposed bodies, where it is not possible to identify the body from DNA or fingerprints. The identification of an individual from skeletal remains involve estimation of age at the time of death, identification of sex, origin and height. Estimation of age at death is one of the basic identifier to find out the true identity of an individual from a decomposed body. Human age estimation at death involves a detail study of bones or fragments of bone of an unknown decomposed body to assign a biological age to that individual.

Human age estimation at death have significant implications in physical anthropology and forensic sciences. In forensic sciences, estimating age at death is used in crime scene investigations (CSI) and disaster victim identification (DVI). In

bio archaeology it can be used for developing demographic profiles. The implications of age estimation at death also lies in paleopathology for identifying the causes and frequency of diseases, which can help in prediction of new diseases or re-emergence of old diseases.

Conventionally, age estimation techniques are based on macroscopic or morphological analysis. Morphological methods involve study of shape and structures of bones. This method focuses on length, diameter, growth, shape and fusion of human bones to identify skeletal maturity (Figure 1.1). Most of morphological age estimation methods rely on the degenerative variations that occurs at the macroscopic level of bone morphology. (Suchey, 1979; Iscan, Loth & Wright, 1984; Katz & Suchey, 1986; Brooks & Suchey, 1990)

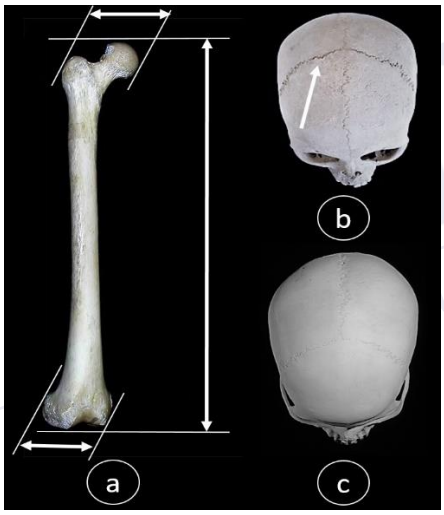
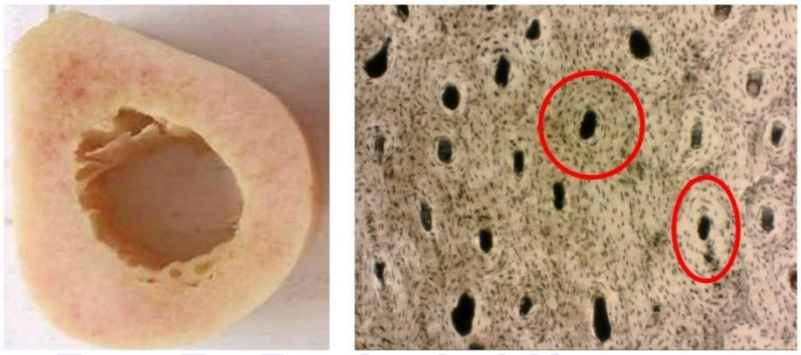


Figure 1.1: Morphological analysis to identify skeletal maturity (a) Long bone (femur) (b) Fusion of skull (c) Mature skull after fusion

Morphological methods are based on subjective assessment of different, and often subtle, stages of degeneration (Ekta, 2017). These methods are non-destructive and can usually be performed quickly, however, they require qualitative analysis that can lead to generate a significant amount of observer error. Morphological age estimation techniques based on identifying the degree of degeneration of bones are imprecise for those beyond 50 years. Moreover, morphological methods of age estimation are not useful in cases of fragmented skeletal remains often encountered in forensic sciences. Subjectivity of morphological or macroscopic observations led to

seek more objective and quantitative methods, based on histological or microscopic analysis of bones.

Histological age estimation methods involve investigation of age-associated variations in microscopic features of bones. These microscopic variations of bones take place due to the life-long metabolic process known as remodeling (Clemente & Miguel, 2018). In these methods observable microscopic features (microstructures) of bones are measured and quantified. These methods are based on the fact that continuous growth and turnover of bones produces distinctive computable microstructures that persist long after their formation. These microstructures are known as osteons or Haversian systems (Figure 1.2), (Stout, 1986; Dudar, Pfeiffer & Saunders, 1993).



(a) (b)

Figure 1. 2: (a) Fragment of femur (b) Microscopic view of femur (Haversian system in red circles)

Histological age estimation methods are advantageous, where the morphology of the skeleton is altered to the degree that morphological methods are no longer applicable, for example, in cases of burned or fragmentary skeletons. Histological methods offer to estimate the age of individuals above 50 years (Ericksen, 1991).

Several methods of age at death estimation from bone histology have been proposed over the years. In these methods age at death have been estimated using skull, ossification centers, dental eruption, epiphyseal closure, length of the long bones and the vertebral column (Cardoso & Rios, 2011; Faridah, Robert & Holger, 2013; Gupta, Kaur, Jawanda & Sahi, 2014; Sinthubua, Theera, Ruengdit & Mahakkanukrauh, 2016; Kacar, Unlu, Beker & Balcik, 2017).

The accuracy of existing histological age estimation methods diverge from each other due to the number and location of samples, bone type, observed microscopic features and amount of fragmented bone required (Katzenberg, 2000). Furthermore, the accuracy of these age estimation methods is dependent on the diagnosis of several age related changes in skeleton that occur during the life span. Different factors such as intrinsic and extrinsic genetic factors, environmental and biomechanical factors alters the histology of the skeleton (Faridah *et al*., 2013). Occupation and different physical activity could also lead to distinct bone mass. Factors such as gender, hormones, disease, diet, life history, ethnicity, nutritional stress and length of daylight influence the rate at which a person's skeleton breaks down (Frame, 1971; Thompson, 1979; Richman, Ortner & Schulter, 1979; Thompson & Gunness, 1981).

Bones with different size of osteons and haversian canals exhibits different osteon population densities and will reach to asymptotes at different stages of age (Pfeiffer, Crowder, Harrington & Brown, 2006). Bone microstructures were also identified to have difference with population and ancestry (Cho, Stout, Madsen & Streeter, 2002; Pfeiffer *et al*., 2006). As bone microstructures show different patterns at same age in two different population due to under or over ageing, it is vital in classifying the reliability of age estimation techniques to consider the variation of bone microstructures with population. Hence, the accuracy of age estimation methods developed on the samples of one population might not be reliable for another population.

Histological age estimation techniques provided relatively accurate results in American and European populations. However, age at death estimation in Malaysian population are mostly based on morphological methods and has not been much benefited from histological age estimation techniques. Histological techniques for age estimation has been addressed by very limited researchers in Malaysian population. Most noticeable histological study for age estimation in Malaysian population is published in 2014 with accuracy of ±10.9 years (Faridah *et al*., 2013). This method was based on manual observation of bone microstructures. Even though histological methods of age estimation has been proven beneficial over morphological methods, yet manual observation of bone microstructures is a time consuming process and can lead to increase the observer error. These limitations could be reduced by automating the process of observation and computation with the help of digital equipment and computer algorithms. This study is dedicated to investigate the characteristics of

REFERENCES

Absolonova, K., Veleminsky, P., Dobisikova, M., Beran, M. & Zocova, J. (2013). Histological Estimation of Age at Death from the Compact Bone of Burned and Unburned Human Ribs. *Journal of Forensic Sciences,* 58(1),pp.135-145.

Adserias, G. J., Zapico, S. (2018). Age Assessment in Forensic Cases: Anthropological, Odontological and Biochemical Methods for Age Estimation in the Dead. *Mathews Journal of Forensic Research,* 1(1), pp. 01-05.

Ahlqvist, J. & Damsten, O. (1969). A modification of Kerley's method for the microscopic determination of age in human bone. *Journal of Forensic Sciences*, 14(2),pp. 205-212.

Aiello, L. C. & Molleson, T. (1993). Are Microscopic Ageing Techniques more accurate than Macroscopic Ageing Techniques? *Journal of Archaeological Science,* 20(6),pp. 689-704.

Albu, I. G. & Georoceanu, R. M. (1990). The canal system in the diaphysial compacta of the femur in some mammals. *Annals of Anatomy,* 170(3),pp.181-187.

Alwyn, M. P. (1983). Dietary risk factors for age-related bone loss and fractures. *The Lancet*, 2(8360),pp.1181-1185.

Alexander, G. R., Sam, D. (2007). Histomorphometry of Human Cortical Bone: Applications to Age Estimation. *Biological Anthropology of the Human Skeleton,* 1(1), pp.149-182.

Beizer, B. C. (1984). Data Systems Analysts. In Pennsauken, Software system testing and quality assurance. New York: Van Nostrand Reinhold Co. pp. 77-83.

Bell, K. L., Loveridge, N., Reeve, J., Thomas, C. D., Feik, S. A. & Clement, J. G. (2001). Super-osteons (remodeling clusters) in the cortex of the femoral shaft: influence of age and gender. *The Anatomical Record*, 264(4),pp. 378-386.

Bertelsen, P. K., Clement, J. G. & Thomas, C. D. L. (1995). A morphometric study of the cortex of the human femur from early childhood to advanced old age. *Forensic Science*, 74(1),pp. 63-77.

Borg, W. R. & Gall, M. D. (1989). An introduction. in Borg, W. R. *Educational research* (5th ed). White Plains, NY: Longman, pp.1-20.

Bouvier, M., Ubleaker, D. H. (1977). A comparison of two methods for the microscopic determination of age at death. *American Journal of Physical Anthropology*, 46(3),pp. 391-394.

Bradbury, S. & Bracegirdle, B. (1998). Introduction to light microscopy. *Microscopy Handbook*. Oxford, UK: Bios Scientific publishers.pp. 37-52.

Britz, H. M., Thomas, C. D., Clement, J. G. & Cooper, D. M. (2009). The relation of femoral osteon geometry to age, sex, height and weight. *Bone*, 45(1),pp.77-83.

Brooks, S. & Suchey, J. M. (1990). Skeletal age determination based on the os pubis: A comparison of the Acsadi-Nemeskeri and Suchey-Brooks methods. *Human Evolution*, 5(3),pp. 227-238.

Burr, D. (2002). Targeted and Nontargeted Remodeling. *Bone*, 30(1),pp.2-4.

Burr, D. B., Ruff, C. B. & Thompson, D. D. (1990). Patterns of skeletal histologic change through time: comparison of an archaic native American population with modern populations. *The Anatomical Record*, 226(3),pp.307-313.

Cardoso, H. F. & Rios, L. (2011). Age estimation from stages of epiphyseal union in the presacral vertebrae. *American Journal of Physical Anthropology*, 144(2),pp. 238-247.

Chan, A. H., Crowder, C. M. & Rogers, T. L. (2007). Variation in cortical bone histology within the human femur and its impact on estimating age at death. *American Journal of Physical Anthropology*,132(1),pp.80-88.

Cho, H., Stout, S. D. & Bishop, T. A. (2006). Cortical bone remodeling rates in a sample of African American and European American descent groups from the American Midwest: comparisons of age and sex in ribs. *American Journal of Physical Anthropology*, 130(2), pp. 214-226.

Cho, H., Stout, S. D., Madsen, R. W. & Streeter, M. A. (2002). Population-specific histological age-estimating method: a model for known African-American and European-American skeletal remains. *Journal of Forensic Science*, 47(1), pp. 12-18.

Clemente, F., Miguel, A. H. & Luis, F. E. (2018). Bone remodeling: A tissue-level process emerging from cell-level molecular algorithms. *Journal Plos One*, 13(9).

Croft, M. A., Glasser, A., Heatley, G., McDonald, J., Ebbert, T., Dahl, D. B., Kaufman, P. L.(2006). Accommodative ciliary body and lens function in rhesus monkeys. *Investigative Ophthalmology & Visual Science*, 47(3),pp.1076-1086.

Cunningham, C., Scheuer, L., & Black, S. (2016). Chapter 3 - Bone Development. In C. Cunningham, L. Scheuer, & S. Black, *Developmental Juvenile Osteology* (Second Edition) San Diego: Academic Press. pp. 19-35.

DeBoer, H. J. A. & Maat, M. (2012). Staining ground sections of natural dry bone tissue for microscopy. *International Journal of Osteoarchaeology*, 22 (4),pp. 379-386.

Deserno, T. M., Antani, S. & Long, R. (2009). Ontology of gaps in content-based image retrieval. *Journal of digital imaging*, 22(2), pp.202-215.

Douglas, H. U. (1974). Reconstruction of demographic profiles from ossuary skeletal samples. *Smithsonia Contributions to Anthropology*, 1(18),pp. 1-79.

Dudar, J. C., Pfeiffer, S. & Saunders, S. R. (1993). Evaluation of morphological and histological adult skeletal age-at-death estimation techniques using ribs. *Journal of Forensic Science*, 38(3), pp. 677-685.

Ekta, P. (2017). Methods of Skeletal Age Estimation used by Forensic Anthropologists in Adults: A Review. *Forensic Research & Criminology International Journal*, 4(2), pp. 01-12.

Ericksen, M. F. (1991). Histologic estimation of age at death using the anterior cortex of the femur. *American Journal of Physical Anthropology*, 84(2), pp. 171-179.

Fangwu, Z. (1983). Preliminary study on determination of bone age by microscopic method. *Acta Anthropologica Sinica* (2), pp. 142-151.

Faridah, M. N., Robert, F. P. & Holger, S. (2013). Age at death estimation from bone histology in Malaysian males. *Medicine, Science and the Law*, 54(4), pp. 203-208.

Frame, B. (1971). The Earlier Gain and the Later Loss of Cortical Bone in Nutritional Perspective. *Radiology*, 98(2), pp. 310-310.

Freemont, A. J. (1998). Bone. *Current Orthopaedics*, 12(3), pp. 181-192.

Frost, H. M. (1962). A model of endocrine control of bone remodeling. *Henry Ford Hospital medical journal*, 10 (1), pp. 19-170.

Frost, H. M. (1982). Mechanical determinants of bone modeling. *Metabolic Bone Disease and Related Research*, 4(4), pp. 217-229.

Frost, H. M. (1983). A determinant of bone architecture :The minimum effective strain. *Clinical Orthopaedics and Related Research*, (175), pp. 286-292.

Frost, H. M. (1985). The “new bone”: Some anthropological potentials. *American Journal of Physical Anthropology*, 28(6), pp. 211–226.

Frost, H. M. (1987). Bone "mass" and the "mechanostat": a proposal. *The Anatomical Record*, 219(1), pp. 1-9.

Gall, M. D., Gall, J. P., & Borg, W. R. (1996). An introduction. in Gall, M. D. *Educational research* (6th ed), Boston: Allyn & Bacon, pp. 1-22.

Gall, M. D., Gall, J. P., & Borg, W. R. (2003). An introduction. in Gall, M. D. *Educational research* (7th ed.), Boston: Allyn & Bacon, pp. 1-17.

Garn, S. M. (1972). The course of bone gain and the phases of bone loss. *Orthopedic Clinics of North America*, 3(3), pp. 503-520.

Greenfield, M. L., Kuhn, J. E. & Wojtys, E. M. (1998). A statistics primer. Correlation and regression analysis. *American Journal of Sports Medicine*, 26(2), pp. 338-343.

Gupta, P., Kaur, H., Shankari, G. S. M., Jawanda, M. K. & Sahi, N. (2014). Human Age Estimation from Tooth Cementum and Dentin. *Journal of Clinical and Diagnostic Research*, 8(4), pp.10.

Hadi, A., Muhammad, M. A. J., Radzi A. & Faridah, M. N. (2018). Bone histology: A key for human sex determination after death. *Journal of Physics:Conference Series*, 1019 (1), pp. 12-19.

Hall, B. K. (2005). Developmental and evolutionary skeletal biology. In Hall, B. K. *Bones and cartilage*, USA: Elsevier Academic Press (Ltd), pp. 1-920.

Heaney, R. P., Recker, R. R. & Saville, P. D. (1978). Menopausal changes in calcium balance performance. *Journal of Laboratory and Clinical Medicine*, 92(6), pp. 953-963.

Iscan, M. Y., Loth, S. R. & Wright, R. K. (1984). Age estimation from the rib by phase analysis: white males. *Journal of Forensic Science*, 29(4), pp. 1094-1104.

Iwaniec, U. T., Crenshaw, T. D., Schoeninger, M. J., Stout, S. D. & Ericksen, M. F. (1998). Methods for improving the efficiency of estimating total osteon density

in the human anterior mid-diaphyseal femur. *American Journal of Physical Anthropology*,107(1), pp.13-24.

Jowsey, J. (1960). Age changes in human bone. *Clinical Orthopaedics*, 17(1), pp. 210-218.

Junqueria, L. (2003). Text and Atlas. Bone. in Junqueria, *Basic Histology,* USA: McGraw-Hill companies, pp. 141-149.

Kacar, E., Unlu, E., Beker, A., Balcik, C., Gultekin, M. A., Kocak, U. (2017). Age estimation by assessing the vertebral osteophytes with the aid of 3D CT imaging. *Australian Journal of Forensic Sciences*, 49(4), pp. 449-458.

Kashi, A. & Saha, S. (2009). Ethics in biomaterials research. *Journal of Long Term Effects of Medical Implants*, 19(1), pp. 19-30.

Katz, D. & Suchey, J. M. (1986). Age determination of the male os pubis. *American Journal of Physical Anthropology*, 69(4), pp. 427-435.

Katzenberg M. A., Ubelaker, D. H. (2000). Methodological considerations in the forensic applications of human skeletal biology. In Ubelaker, D. H. *Biological anthropology of the human skeleton*, New York: Wiley-Liss, pp. 41–67.

Keough, N. (2007). *Estimation of age at death from the microscopic structure of the femur.* University of Pretoria, School of Medicine: Master's Thesis.

Kerley, E. R. (1965). The microscopic determination of age in human bone. *American Journal of Physical Anthropology*, 23(2), pp. 149-163.

Lanyon, L. E., Sugiyama, T. & Price, J. S. (2009). Regulation of bone mass: Local control or systemic influence or both?. *BoneKEy*, 6(6), pp. 218-226.

Leeson, R. C., Leeson, T. S. & Paparo, A. A. (1985). Specialized connective tissue:cartilage and bone. In Leeson, R. C. *Textbook of Histology*, 5 edn, Japan: W.B. Saunders Co, pp. 125-149.

Lieberman, D. E., Polk, J. D., & Demes, B. (2004). Predicting long bone loading from cross-sectional geometry. *American Journal of Physical Anthropology*, 123(2), pp. 156-171.

Maat, G. J., Maes, A., Aarents, M. J., & Nagelkerke, N. J. (2006). Histological age prediction from the femur in a contemporary Dutch sample. The decrease of nonremodeled bone in the anterior cortex. *Journal of Forensic Science*, 51(2), pp. 230-237.

Maat, G., Robert, P.M., Aarents, M. j. (2001). Manual preparation of ground sections for the microscopy of natural bone tissue: Update and modification of Frost's

rapid manual method. *International Journal of Osteoarchaeology*, 11(1), pp. 366 - 374.

Mansourvar, M. (2014). *Bone age assessment using hand and clavicle X-ray images*. University of Malaya: Phd Thesis.

Martin, B. R. & Lovell, N. C. (1990). Structure function, and adaptation of compact bone. *American Journal of Physical Anthropology*, 82(1),pp. 116-117.

Mayette, S. & Emily Amor, B. (2013). Empirical power comparison of goodness of fit tests for normality in the presence of outliers. *Journal of Physics*, 435(1), pp. 12-41.

McDonald, X. F. & Fen, X. (2011). Disorders of Bone Remodeling. *Annual Review of Pathology:* 6 (1), pp.121-145.

Miszkiewicz, J. (2016). Investigating histomorphometric relationships at the human femoral midshaft in a biomechanical context. *Journal of Bone and Mineral Metabolism*, 34(2), pp. 179- 192.

Nor, F. M., Pastor, R. F., & Schutkowski, H. (2014). Age at death estimation from bone histology in Malaysian males. *Medicine, Science and the Law*, 54(4), pp. 203-208.

Ott, S. (2002). *Histomorphometric analysis of bone remodeling*. in Bilezikian, J. Raisz, L., Rodan, G (Ed). Principles of Bone Biology, United States: Academic Press, ,pp. 303-320.

Paine, R. R., & Brenton, B. P. (2006). Dietary health does affect histological age assessment: an evaluation of the Stout and Paine (1992) age estimation equation using secondary osteons from the rib. *Journl of Forensic Science*, 51(3), pp. 489-492.

Pfeiffer, S. (1998). Variability in osteon size in recent human populations. *American Journal of Physical Anthropology* 106(2), pp. 219-227.

Pfeiffer, S., Crowder, C., Harrington, L., & Brown, M. (2006). Secondary osteon and Haversian canal dimensions as behavioral indicators. *American Journal of Physical Anthropology*, 131(4), pp. 460-468.

Pollitzer, W. S. & Anderson, J. J. (1989). Ethnic and genetic differences in bone mass: a review with a hereditary vs environmental perspective. *The American Journal of Clinical Nutrition*, 50(6), pp. 1244-1259.

Raggatt, L. J., (2010). Cellular and Molecular Mechanisms of Bone Remodeling. *The Journal of Biological Chemistry*, 285(233), pp. 25103- 25108.

Rzali, M. & Yap, B. (2011). Power Comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling Tests. *Journal of Statistical Modeling and Analysis*, 2(1), pp. 21-33.

Richman, E. A., Ortner, D. J. & Schulter, F. P. (1979). Differences in intracortical bone remodeling in three aboriginal American populations: possible dietary factors. *Calcified Tissue International*, 28(3), pp. 209-214.

Robling, A. G. & Stout, S. D. (2007). Histomorphometry of Human Cortical Bone: Applications to Age Estimation. in Robling, A. G. *Biological Anthropology of the Human Skeleton* (2nd Ed), New Jersey: John Wiley & Sons, pp. 149-182.

Ross, M. H., Romrell, L. J., Kaye, G. I. (1995). Bone. in *Histology*: *A Text and Atlas*,(3Ed). Philadelphia: Williams & Wilkens, pp. 150-187.

Samson, C. B. (1987). A new method of estimating age at death from fragmentary and weathered bone. In Bodington, A. & Garland, A. N. *Death Decay and Reconstruction Approaches to Archaeology and Forensic Science*. Manchester: Manchester University Press, pp. 101-108.

Singh, I. J., & Gunberg, D. L. (1970). Estimation of age at death in human males from quantitative histology of bone fragments. *American Journal of Physical Anthropology*, 33(3), pp. 373-381.

Sinthubua, A., Theera, N., Auephanwiriyakul, S., Ruengdit, S., Das, S. & Mahakkanukrauh, P. (2016). New Method of Age Estimation from Maxillary Sutures Closure in a Thai Population. *Journal of Clinical Therapeutics*, 167(2), pp. 33-37.

Slayter, E. M. & Slayter, H. S. (1992). Light and electron microscopy. In Slayter, E. M. *The Principles and Practice of Electron Microscopy*, UK:Cambridge University Press.

Stevenson, P. H. & Steyn, M. A. (1998). Age order of epiphyseal union in man. Sexual dimorphism in the crania and mandibles of South African whites. *American Journal of Physical Anthropology*, 7 (1), pp.53-93.

Stout, S. D., & Gehlert, S. J. (1980). The relative accuracy and reliability of histological aging methods. *Journal of Forensic Sciences*,15(3), pp. 181-190.

Stout, S. D. (1986). The use of bone histomorphometry in skeletal identification: the case of Francisco Pizarro. *Journal of Forensic Sciences*, 31(1), pp. 296-300.

Stout S. D. (1989). Histomorphometric analysis of human skeletal remains. in Iscan M. Y., Kennedy, K.A. *Reconstruction of Life from the Skeleton*. New York: Alan R.Liss,pp. 41-52.

Stout, S. D., & Paine, R. R. (1992). Histological age estimation using rib and clavicle. *American Journal of Physical Anthropology*,87(1), pp.111-115.

Stout, S. D., Dietze, W. H., Iscan, M. Y. & Loth, S. R. (1994). Estimation of age at death using cortical histomorphometry of the sternal end of the fourth rib. . *Journal of Forensic Sciences*, 39(3), pp. 778-784.

Suchey, J. M. (1979). Problems in the aging of females using the Os pubis. *American Journal of Physical Anthropology*, 51(3), pp. 467-470.

Teitelbaum, S. L. (2000). Bone resorption by osteoclasts. *Science*, 289(5484), pp. 1504-1508.

Tersigni, M.A. (2005). *Serial long bone histology: Inter- and intro-bone age estimation*, University of Tennessee: PhD Thesis.

Thompson, D. (1979). The Core Technique in the Determination of Age at Death in Skeletons. *Journal of Forensic Sciences*, 24(4), pp. 902-915.

Thomas, C. D., Feik, S. A. & Clement, J. G. (2005). Regional variation of intracortical porosity in the midshaft of the human femur: age and sex differences. *Journal of Anatomy*, 206(2), pp. 115-125.

Thomas, C. D. L., Stein, M. S., Feik, S. A., Wark, J. D. & Clement, J. G. (2000). Determination of age at death using combined morphology and histology of the femur. *Journal of Anatomy*, 196(3), pp. 463-471.

Thompson, D. (1981). Microscopic Determination of Age at Death in an Autopsy Series. *Journal of Forensic Sciences*, 26 (3), pp. 470-475.

Thompson, D. D., & Gunness, M. (1981). Bone mineral-osteon analysis of Yupik-inupiaq skeletons. *American Journal of Physical Anthropology*, 55(1),pp. 1-7.

Watanabe, Y., Konishi, M., Shimada, M., Ohara, H. & Iwamoto, S. (1998). Estimation of age from the femur of Japanese cadavers. *Journal of Forensic Sciences*, 98(1-2), pp. 55-65.

Weinstein, R. S., & Bell, N. H. (1988). Diminished rates of bone formation in normalblack adults. *Journal of Medicinal Chemistry*, 319(26), pp.1698-1701.

White, T. D., Black, M. T., & Folkens, P. A (2012). Skull. In Black. *Cranium and Mandible Human Osteology* (3rd Ed). San Diego: Academic Press, pp. 43-100.

Wit, B. B. (1997). Catch-up Growth. *Endocrine Reviews*, 18(5), pp. 646–661.

Wu, K., Schubeck, K. E., Frost, H. M. & Villanueva, A. (1970). Haversian bone formation rates determined by a new method in a mastodon, and in human diabetes mellitus and osteoporosis. *Calcified Tissue International*, 6(3),pp. 204-219.

Yoshino, M., Imaizumi, K., Miyasaka, S. & Seta, S. (1994). Histological estimation of age at death using microradiographs of humeral compact bone. *Forensic science*, 64(2-3), pp.191-198.